#18

Department of Agriculture
and Fisheries for Scotland

Changes in the Respiration and Blood Circulation of Cod, *Gadus morhua L.*, Induced by Exposure to Pollutants

A D F Johnstone
A D Hawkins

JAN 9

Scottish Fisheries
Research Report
Number 18 1980
ISSN 0308-8022

Changes in the Respiration and Blood Circulation of Cod, *Gadus morhua L.*, Induced by Exposure to Pollutants

ADF Johnstone AD Hawkins

Marine Laboratory Aberdeen

Introduction

Many toxicants inhibit oxygen uptake by the gills of fish (Skidmore, 1970; Abel, 1974), cause variation in oxygen consumption (Calabrese, Thurberg, Dawson and Wenzloff, 1975) and may also change ventilatory acitivity (Sellers, Heath and Bass, 1975). Some toxicants interfere directly with blood chemistry (Dively, Mudge, Neff and Anthony, 1977; Zeitoun, 1977), blood circulation (Holeton, 1971; Hughes and Adeney, 1977a) or key metabolic enzymes of fish (Brown, 1976), and may hinder oxygen uptake by the tissues.

Experiments on cod were conducted to examine the effects of a range of potentially damaging substances on heart rate, gill ventilation rate and amplitude, and oxygen uptake. The test substances were cryolite recovery sludge (an aluminium smelter waste), an oil dispersant (BP 1100 WD), crude oil from the North Sea (Forties Field), and a mixture of crude oil and dispersant in sea water. The intention was to assess the value of various metabolic parameters as early indicators of physiological damage to the fish.

Materials and Methods

The juvenile cod used were one to two years old, from 29 to 45 cm long, and weighing from 179 to 440 g, and were captured on baited lines at depths of less than 10 m in Upper Loch Torridon, Ross-shire, on the west coast of Scotland.

Some experiments were performed in a recirculating aquarium at the Marine Laboratory in Aberdeen during 1976, but most were conducted on site during 1977 in a small aquarium with water drawn directly from the loch. Sea water temperatures ranged from 9.0 to 14.0° C, salinity from 32.0 to 33.0‰, and pH from 8.0 tp 8.5.

The fish were allowed to settle, unfed, in fibreglass tanks supplied with clean sea water for at least one week after capture. Individuals damaged during capture and subsequent handling were rejected. Each selected individual was anaesthetised with a 1:10,000 solution of MS222 (Sandoz) (tricaine methane sulphonate) in sea water until ventilation ceased. A subcutaneous electrocardiograph electrode inserted ventrally was retained by a suture. The electrode consisted of two fine (0.27 mm diameter) stainless steel wires, insulated with diamel, and bared at the tip. One wire was 3 mm longer than the other (Fig. 1). Each wire was soldered to an insulated stranded copper wire, and the joint covered with a quick setting epoxy resin and shrink-fit sleeving.

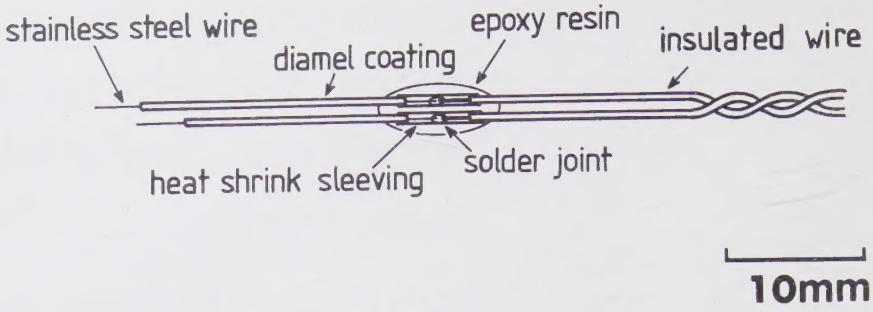

Figure 1. Electrocardiograph electrode for the experiments.

After insertion of the electrode the fish was allowed to recover from the anaesthetic in the experimental chamber, continuously supplied with fresh, aerated sea water (Fig. 2). A ground electrode of stainless steel mesh was placed on the floor of the tank.

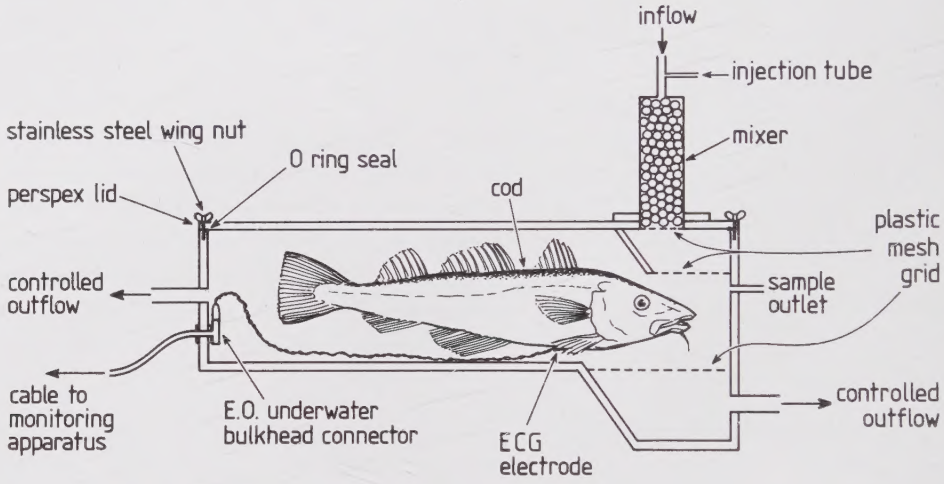

Figure 2. The perspex fish chamber in side view.

The signal from the inserted electrode was fed to two separate amplifiers (Tektronix, type 122). One, with a bandwidth of 50 to 80 Hz, filtered out the lower frequency respiratory muscle potentials and yielded the electrocardiogram (ECG), whilst the other, with a bandwidth of 0.2 to 50 Hz, indicated the ventilatory movements of the fish. A block diagram of the monitoring equipment is given in Figure 3. Both signals were displayed on a two channel pen recorder (Devices MX212) together with a clock signal and event marker. The ECG signal was also fed to a monitoring system which printed out the duration of each heart-beat interval, or a sequence of heart-beat intervals at pre-set times.

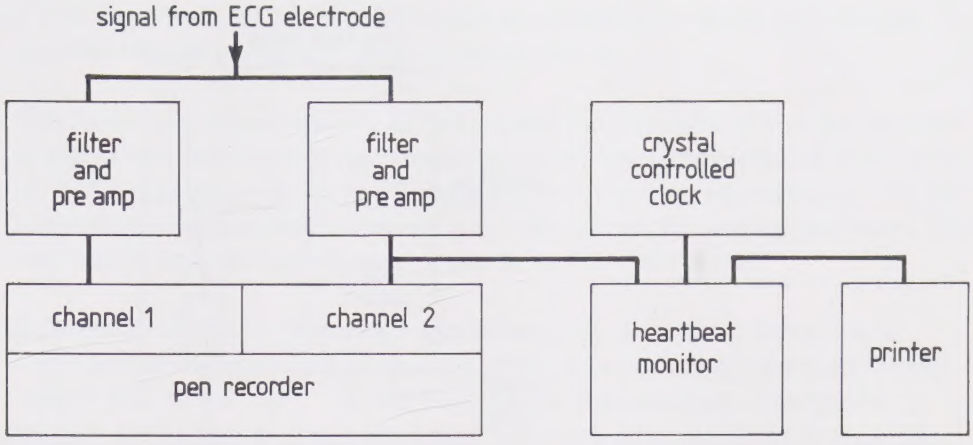

Figure 3. Block diagram of the apparatus for monitoring the heart and respiration rates.

In the experiments the fish chamber formed part of an open circuit (Fig. 4). In this system the fish was supplied with water of known temperature and oxygen content. After passing through the fish chamber the oxygen concentration, temperature, salinity and pH were measured. The oxygen consumption of the fish was calculated and expressed as mg of O_2/kg/h, where kg = live weight.

The oxygen content of the aerated inflowing water was measured with a lead anode oxygen electrode (EIL type 15A) to an accuracy of ± 5%, and temperature by a thermistor probe to an accuracy of ± 1.5° C. The oxygen content of the outflowing water was monitored with a polarographic electrode (Beckman type 39552) to an accuracy of ±1%, temperature and salinity by means of a T/S bridge (EIL type MC5) to an accuracy of ±0.05‰ for salinity and ±0.1° C for temperature. pH was measured with a portable electrode system (Albert Measurements Limited). All the tanks in the circulatory system were of thick polythene or clear acrylic plastic (perspex). The tubing was of polyvinyl chloride or of silicone rubber coated with vaseline grease to reduce gaseous exchange.

The oxygen electrodes were calibrated before and after each experiment by alternately placing them in aerated sea water of known salinity and temperature, and sea water through which oxygen-free nitrogen had been bubbled. The electrodes were occasionally checked by taking water samples from inflow and outflow and determining the oxygen content by the Winkler method.

Flow rate of the system was measured by collecting the outflowing sea water and determining the volume for a known period of time (ml per minute) to an accuracy of ±2.0%.

The experimental animals were normally left to settle for 24 hours after introduction to the chamber, and the resting levels of the various metabolic parameters taken as the mean value over the period preceding the start of an experiment (usually one hour).

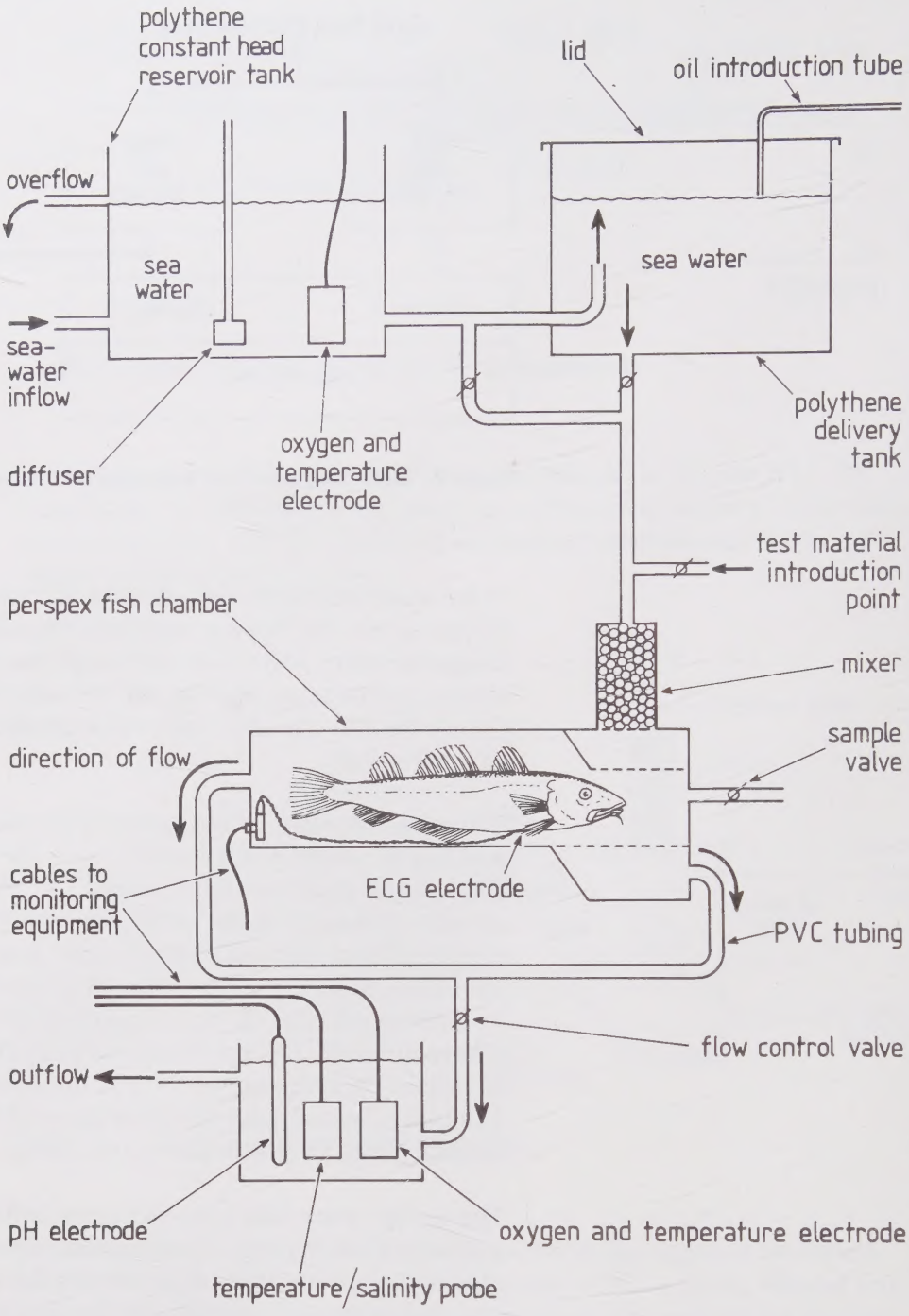

Figure 4. The open circuit sea water system showing the tank employed for the administration of crude oil. Valves in the open system permit the delivery tank to be by-passed.

Concentrations of test materials at the head of the fish during presentation were extrapolated from a calibration curve determined with a water soluble dye and a spectrophotometer.

The following experiments were performed with a resting fish in the chamber: A suspension of cryolite recovery sludge was injected from either of two samples. The sludge was a black suspension of about 10% solids, mainly alumina and carbon, in a complex, dilute aqueous waste (details of composition are given in the Appendix).

The sludge was continually pumped in its own closed circuit from a stirred reservoir by means of a peristaltic pump. When injection into the inflow to the fish chamber was desired the sludge was diverted into the water supply to the fish by opening a silent valve. Later, a filtrate of the sludge was employed as the stimulant.

Three experimental animals were each presented with whole and filtered cryolite recovery sludge in separate experiments.

The maximum concentration of test material at the head of the fish during presentation was the original concentration of the sample diluted by a factor of 0.005 and occurred 15 to 20 seconds after injection commenced. The sea water flow rate was maintained at 2.5 l per minute for each experiment. The respiratory and cardiac changes of the three fish were noted.

A quantity of the oil dispersant was injected by peristaltic pump into a mixing chamber upstream of the fish. The concentrations presented ranged from 4,500 to 4.7 mg l^{-1} at the front of the fish chamber. Changes in oxygen consumption, respiration and heart rate were recorded in separate experiments with five cod.

One litre of crude oil, previously stored in a sealed container to retain volatile components, was pumped onto the surface of a tank situated between the constant head reservoir tank and the fish chamber (Fig. 4). The oil formed a layer 10 mm thick on top of the sea water. The water supply to the fish passed beneath the layer of oil at a known rate (0.34 l per minute).

Changes in oxygen consumption, ventilation and heart rate were recorded in separate experiments on two fish.

A further experiment was undertaken to test the direct effect of an oil/water dispersion without introducing an oil slick to the circulatory system. 20 ml of crude oil was mixed vigorously with three litres of sea water in a sealed container for 60 minutes by a magnetic stirrer. The resulting suspension was allowed to settle for 15 minutes. The aqueous extract was then injected into the mixing chamber upstream of the fish. The test material was introduced at a rate of 27 ml per minute for a period of 90 minutes into water flowing at a rate of 0.36 l per minute. The material was diluted by a factor of 0.074 before reaching the anterior of the fish chamber. Changes in oxygen consumption, ventilation and heart rate were monitored.

To examine the effect of crude oil and dispersant together, an experiment was conducted in a similar manner to that described for the oil-water dispersion alone. 30 ml of crude oil was added to three litres of dispersant solution at a concentration of 1 g l^{-1} in sea water and mixed vigorously for 60 minutes. Before injection of the resulting oil/dispersant/sea water suspension, mixing was stopped for 15 minutes allowing most of the non-emulsified oil to return to the surface of the mixture. Only the aqueous part of the mixture was used in the experiment on one cod.

The test mixture was introduced at a rate of 27 ml per minute for 90 minutes into water flowing at a rate of 0.4 l per minute. The original concentration was diluted by a factor of 0.071 by the time the test material reached the anterior of the fish chamber. Changes in respiration and cardiac rate of the experimental animal were noted.

Results

Respiratory and cardiac changes in the presence of cryolite recovery sludge

The first fish tested showed no change in heart rate when presented with whole sludge (Sample 1) or filtrate (Sample 1). Ventilatory activity was not monitored since the opercular beats were low in amplitude and difficult to detect. The second fish showed no change in heart rate during or after presentation of whole sludge (Sample 1) but several irregular high amplitude changes in ECG were recorded from 25 to 50 seconds after introduction of the same test material. An observer watched subsequent presentations and noted that the fish coughed following the introduction of the sample of

sludge (Fig. 5a). The changes in ECG probably represented sudden violent movement of the ECG electrode and the generation of additional muscle potentials. Coughing consisted of abnormally large movements of the mouth and opercula, suggesting that respiratory intake was increased or that respiratory flow was temporarily reversed (Sellers *et al.*, 1975). The filtrate (Sample 1) did not elicit a response in the form of a change in the rate of ECG or ventilatory activity. A third fish also responded to presentation of whole cryolite sludge (Sample 2) with a coughing response as shown in Figure 5b. Coughing was usually associated with a pronounced slowing of the heart rate for one or more beats (bradycardia). No response to the filtrate was obtained.

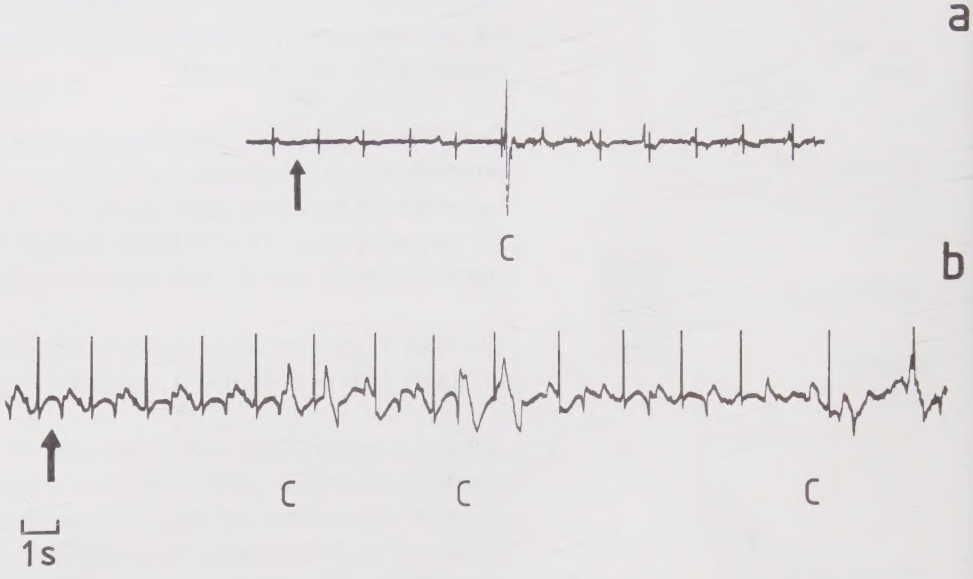

Figure 5. Recordings of ECG and gill movement potentials from two cod subjected to administration of cryolite recovery sludge.
a and b: Coughing responses elicited after injection of whole sludge demonstrating the bradycardia sometimes associated with the response. (The bandwidth of the preamplifier filter was altered during recording of the signal shown in b to display ventilatory movements more clearly.)
C = coughing response. ↑ **indicates the end of the 10 second period of injection of the test material.**

Respiratory and cardiac changes in the presence of the oil dispersant

Of the five concentrations of dispersant investigated the highest concentration (4,500 mg l^{-1}) proved fatal to the experimental animal after 35 minutes exposure. A concentration of 500 mg l^{-1} proved lethal after 90 minutes exposure. The three remaining fish survived exposure at lower concentrations for 90 minutes and thereafter returned to steady pre-introduction levels of oxygen consumption, heart and ventilation rate, and subsequently survived in apparently healthy condition for at least five days. The oxygen consumption, heart and ventilation rates for each fish presented with dispersant are shown in Figure 6a, b and c.

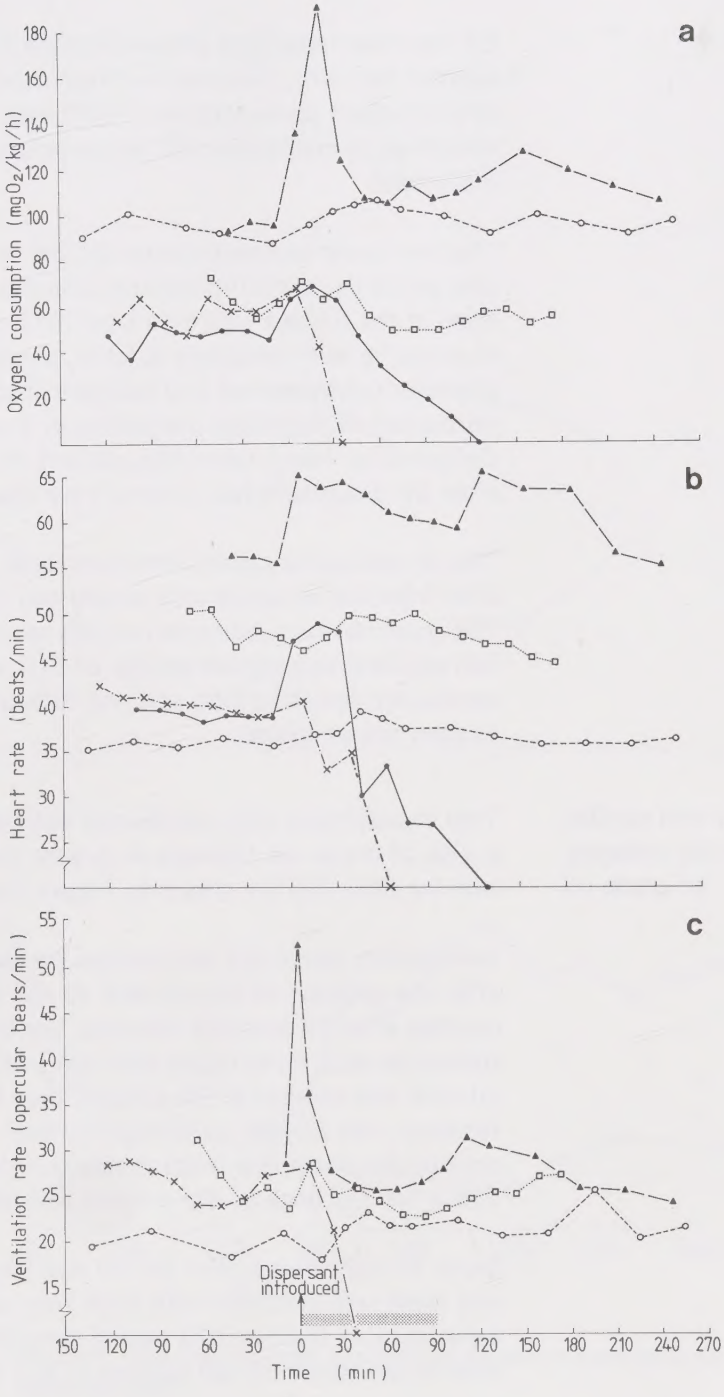

Figure 6. The results of experiments to test the effects of an oil dispersant (BP 1100 WD) on cod showing:

a oxygen consumption

b heart rate and

c ventilation rate for each fish before, during and after introduction of a dispersant concentration in the fish holding tank for 90 minutes.

Note that ventilation rate was not recorded during the presence of the 500 mg l^{-1} dispersant concentration.

Dispersant concentrations and fish weights:

x = 4,500 mg l^{-1}, 382 g

▲ = 210 mg l^{-1}, 263 g

O = 4.7 mg l^{-1}, 440 g.

● = 500 mg l^{-1}, 304 g

□ = 47 mg l^{-1}, 316 g

The two highest concentrations of dispersant (4,500 and 500 mg l^{-1}) resulted in an increase in heart and ventilation rate and a corresponding increase in oxygen consumption. The stronger concentration caused only a brief response (increased heart and ventilation rate) before death, the weaker concentration eliciting a more prolonged reaction as the fish survived for longer.

Of the three remaining concentrations tested (210, 47 and 4.7 mg l^{-1} at the head of the fish), the most marked response occurred with the introduction of the highest concentration. Heart rate, ventilation rate and oxygen consumption immediately rose to maximum values on introduction of the dispersant.

The two lower concentrations elicited only slight responses, the ventilation rate giving the most immediate indication of the presence of the dispersant. Even at the highest tolerable level (210 mg l^{-1}) the ventilation rate returned to a resting level relatively quickly, some 40 minutes after introduction of dispersant commenced and before introduction ceased altogether, the concentration of dispersant remaining at the test level for a further 50 minutes. On the other hand, heart rate did not return to a resting level until two hours after the dispersant had cleared from the fish chamber.

The physiological parameters monitored sometimes increased in level just after injection of dispersant ceased and the fish holding chamber cleared. The most obvious indication of this secondary response was displayed by the fish exposed to a concentration of 210 mg l^{-1} of dispersant. Increased ventilation occurred first and was followed by an increase in heart rate and oxygen consumption.

Respiratory and cardiac changes in the presence of crude oil

Two experiments were conducted with water flowing from a tank containing a slick of crude oil. Changes in oxygen consumption, heart and ventilation rate for both fish are shown in Figure 7a, b and c.

Ventilation, heart rate and oxygen consumption for both fish increased after the addition of the oil slick to the system. Maximum values were reached after 67 and 105 minutes. Ventilation and heart rate decreased thereafter and, three hours after the introduction of the oil and with the oil slick still present in the system, they had fallen to the original levels. However, the oxygen consumption level of the 244 g fish remained continually above the original rate but the oxygen consumption level of the 218 g fish fell back to the original rate after three hours.

Seven to eight hours after the oil was introduced, a second rise in ventilation and heart rate occurred with both fish, perhaps indicating that a particular water soluble fraction had passed through to the fish chamber. (The outflowing sea water turned opaque at this time.)

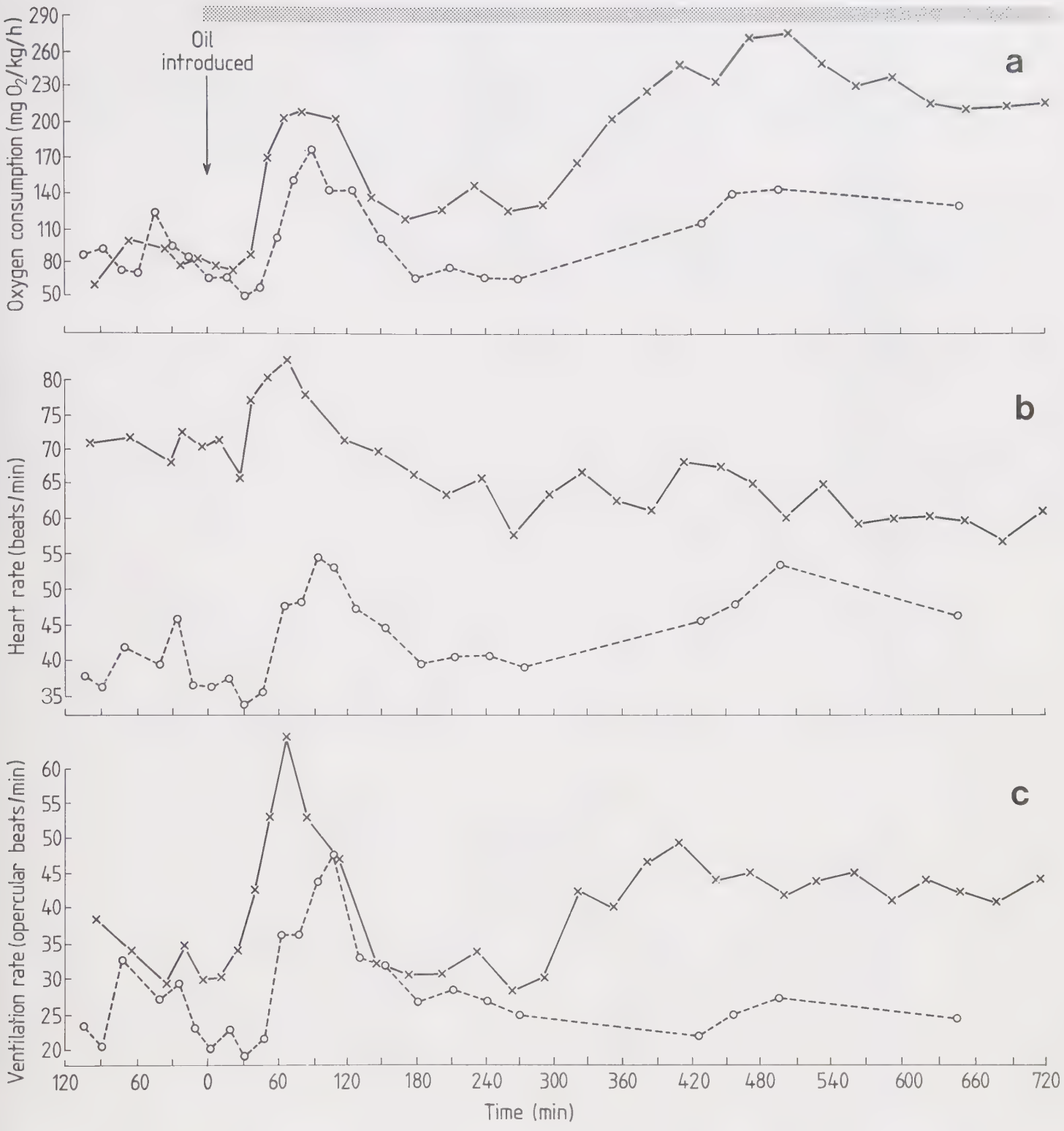

Figure 7. The results of two experiments on cod to investigate the effects of a simulated crude oil slick.

a oxygen consumption

b heart rate and

c ventilation rate for each fish before and during the presence of the oil.

Fish weights: 0 = 218 g x = 244 g.

One experiment was carried out to test the effect of an oil/water dispersion (OWD). Changes in the oxygen consumption, heart and ventilation rate for a 33 cm cod weighing 286 g in the presence of the OWD are shown in Figure 8a, b and c. Oxygen consumption rose to a maximum within 25 minutes of the introduction of the OWD and only settled to resting level 30 minutes after injection ceased. Ventilation and heart rates rose to maximum rates 10 minutes after introduction and fell back to the resting levels just after the injection of the OWD stopped.

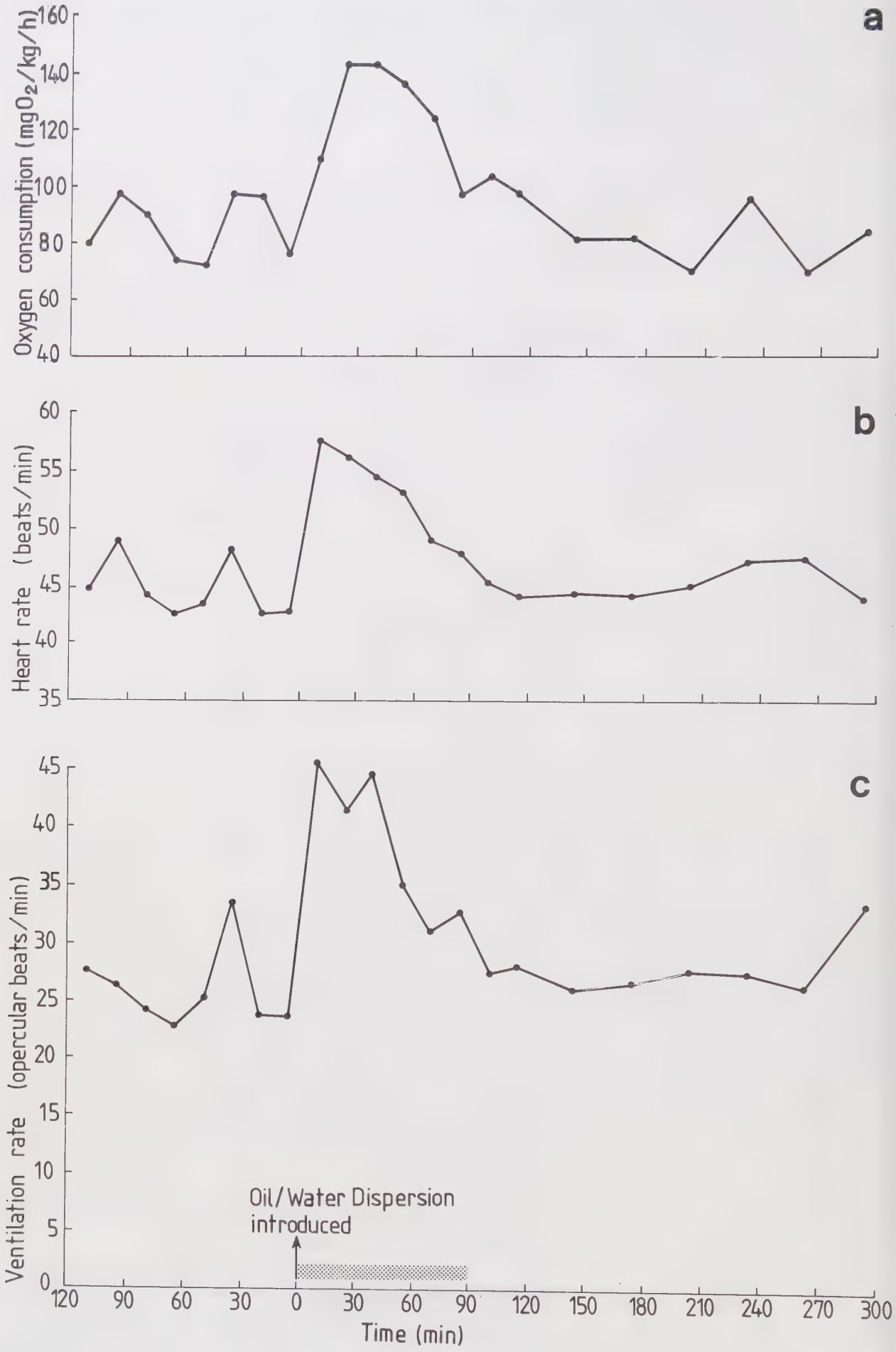

Figure 8. The results of an experiment on the effects of an oil/water dispersion (OWD) on a cod of 286 g weight.

a oxygen consumption

b heart rate and

c ventilation rate of the fish before, during and after administration of the OWD.

The three fish subjected to the presence of crude oil or the OWD survived exposure and were in apparently healthy condition five days later.

Respiratory and cardiac changes in the presence of a mixture of crude oil and dispersant

One experiment was performed using a mixture of crude oil and dispersant in sea water. The approximate concentration of dispersant in the anterior of the chamber was 70.0 mg l^{-1} with the addition of emulsified oil and oil droplets present at an unmeasured level.

The oxygen consumption of the fish rose to a peak immediately after introduction of the test material. The maximum oxygen consumption occurred 80 minutes after injection of the test material and fell once injection ceased but had not reached the previous resting level three hours later (Fig. 9a).

Heart and ventilation rates (Fig. 9b and c) rose immediately after introduction of the test material. The ventilation rate remained permanently above the resting rate, reaching a peak just before injection stopped.

Heart rate showed a brief drop 20 minutes after introduction of the mixture, but then increased just before the injection ceased. After injection stopped the rate dropped to around resting level but increased again and still remained above resting rate three hours later. However, the fish survived exposure to the mixture and appeared to be in a healthy state five days later.

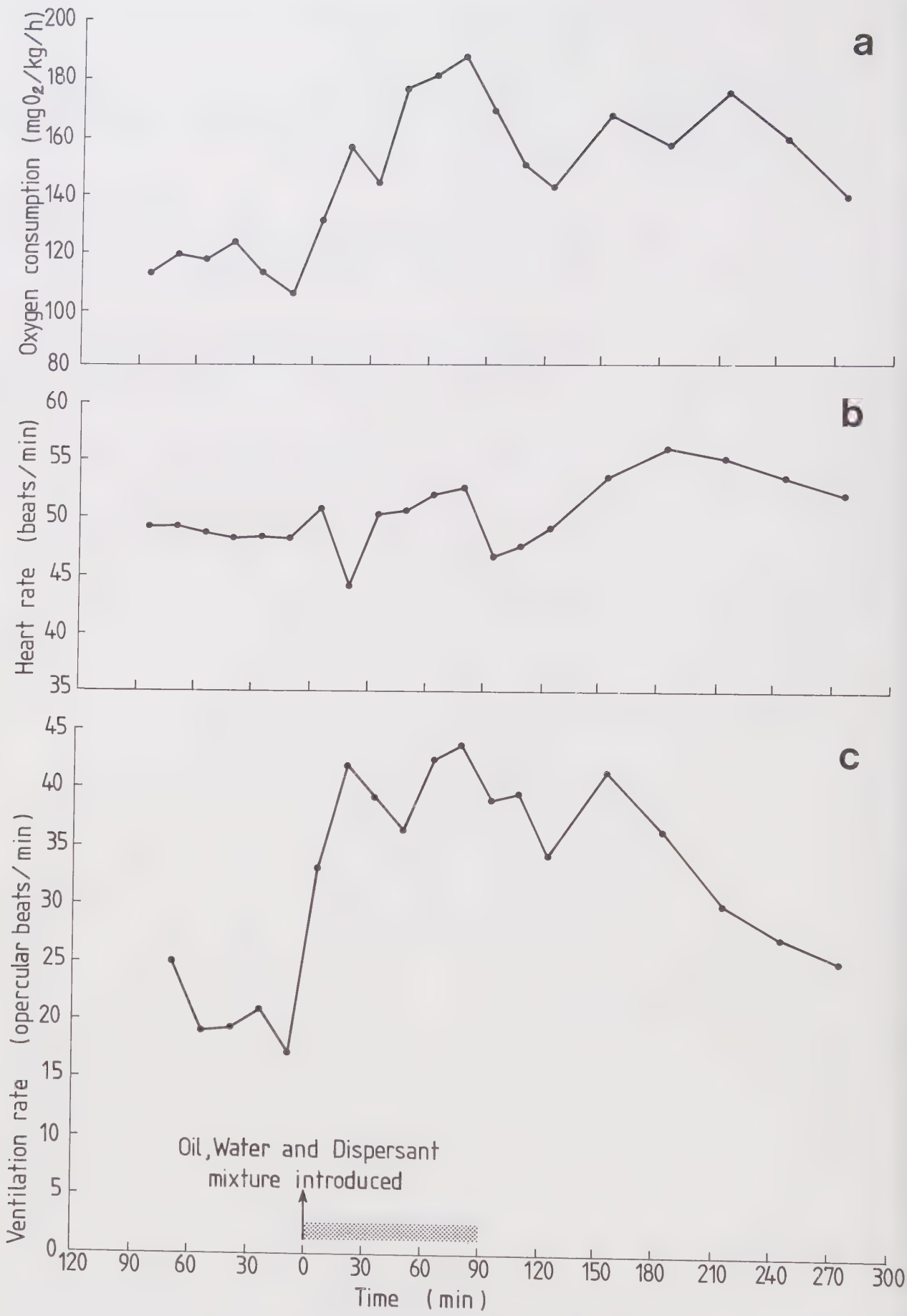

Figure 9. The results of an experiment to investigate the effects of an oil/dispersant/sea water mixture on a cod of 170 g weight.

a oxygen consumption

b heart rate and

c ventilation rate of the fish before, during and after presentation of the mixture.

Discussion

The effects of cryolite recovery sludge

Coughing responses, which are a normal part of the respiratory activity of most fishes (Hughes and Adeney, 1977b), may result from direct stimulation of the gills by a range of substances, including heavy metal salts (Schaumberg, Howard and Walden, 1967; Skidmore, 1970). Hughes (1975) found that rainbow trout exhibit changes in coughing frequency when exposed to suspended wood pulp fibre in circulated water. It has been suggested that the increased coughing rate following the exposure of fish to "kraft effluent" results from hypoxia (Schaumberg *et al.*, 1967) but we have not observed a direct coughing response during observations on the effects of hypoxia on cod (Johnstone and Hawkins, unpublished observations). Coughing can be elicited in rainbow trout by the addition of dyes to the water (Hughes and Adeney, 1977b) and has been suggested as a useful response for monitoring water quality (Lunn, Toews and Pree, 1976; Carlson and Drummond, 1978).

In our experiments a coughing response was only elicited from cod in the presence of suspended sludge. Van Dam (1938) suggested that such a response may be an attempt to rid the gills of foreign material. In our experiments no changes in heart rate occurred prior to the coughing response and filtered sludge caused no coughing, which suggests that the suspended matter was probably detected by mechanoreceptors in the area of the gills. Cryolite recovery sludge had no other observable effect upon the cardiac and respiratory physiology of the fish, though it may well affect the behaviour of fish in some other respect.

The effects of oil dispersant

It has been suggested by many authors that the emulsifiers used in clearing up oil pollution at sea are often more dangerous to marine organisms than the oil itself (eg Lønning, 1977). Kiceniuk, Penrose and Squires (1978) found that dispersants caused bradycardia in the marine fish, *Tautogolabrus adspersus.* However, in our experiments with cod the presence of dispersant caused an increase in heart rate. During our experiments with dispersant, ventilation rate gave the most obvious indication of the presence of the material although the rise in ventilation rate was not prolonged and returned to resting level while dispersant was still present in the system. The lowest concentration of dispersant in our experiments was 4.7 mg l^{-1} which elicited only a slight response. Recent unpublished investigations by one of us (Johnstone) into the chemosensory sensitivity of cod have shown that their threshold of detection for the dispersant BP 1100 WD, which is one of the 'low toxicity' dispersants, is around 0.3 mg l^{-1} of the concentrate. It would appear, therefore, that juvenile and adult cod can detect and may react to dispersant concentrations below those which cause immediately observable metabolic changes.

Apart from any direct toxic effects of oil dispersants their action when applied to oil at sea is to reduce the interfacial tension between oil and sea water, which promotes droplet formation. The surface active molecules present in the chemical dispersant become located at the oil/sea water interface and prevent coalescence of the droplets once formed. Dispersant chemicals cause the initial concentrations of oil in the upper 0.3 m of water to be higher than for naturally dispersed oil which disperses at a slower rate (Cormack and Nichols, 1977). It has been suggested that oil dispersions will only reach toxicity level (LC 50) when they have been dispersed by means of chemicals (Kühnhold, 1977). According to Cormack and Nichols (1977) the most satisfactory method of dealing with oil slicks which are threatening coastal areas is by the use of suitable emulsifying chemicals. The normal application rate of dispersant for oil slicks at sea is 1:3 by volume (where the concentrate dispersant is diluted 1:10 with sea water before application). In our experiment testing the combined effect of dispersant and oil, the ratio of concentrate dispersant to oil was 1:10 before presentation to the fish.

Comparing the effects of the maximum tolerable concentration of dispersant alone (210 mg l^{-1}) with the effects of the dispersant/oil mixture it can be seen that the latter elicited a more marked and long lasting response from the fish, even though the actual dispersant concentration was much lower (70 mg l^{-1}). It can be inferred, then, that exposure to a dispersant/oil mixture causes a greater change in the physiological state of fish.

The effects of oil

The effects and toxicity of the water soluble fraction of crude oil on marine organisms have been reported by several authors (eg Anderson, Neff and Petrocelli, 1974; Thomas and Rice, 1975) and a critical assessment of published data on the effects of oil on marine organisms is given by Moore and Dwyer (1974) and its effect on fisheries by Johnston (1977). Recent researchers on the effects of oil have used fish eggs and larvae as experimental organisms (Lønning, 1977; Kühnhold, 1977), since these are believed to be more sensitive to oil pollution than adult organisms. The direct effects upon commercially valuable fish species are most likely to occur in the event of an oil spillage in a confined area, such as a sea loch, where fish are more confined than in the open sea. Our results demonstrate that the direct effects upon circulation and respiration produced by subjecting cod to a supply of sea water flowing under a new oil slick are generally not of long duration, the fish returning to its resting state after three hours of exposure. The oil/water dispersion, presented on its own, gave similar results.

It is difficult to relate the results of laboratory experiments to the effects of oil occurring in the sea (Johnston, 1977) but our results give some indication of the sub-lethal short term effects of oil on some physiological parameters of fish. Since respiration rate is an easily measured indicator of the metabolic state of the fish, our technique can be used as a short term test for acute toxicity. Monitoring respiration rate has been suggested recently as a useful method of monitoring the effects of toxicants by several authors (Sparks, Cairns and Heath, 1972; Morgan and Kühn, 1974; Thomas and Rice, 1975). However, Sprague (1971) was dubious about the significance of change in rate as a response to a toxicant but stated that 'it might be useful in some circumstances as a sign of stress in fish'.

The range of short term toxicity of crude oil to marine fish is widely reported as 10^4 to 10^5 mg l^{-1} (Johnston, 1977) when the fish are maintained in water directly beneath an oil slick in conditions similar to a fresh spill of North Sea crude oil at the sea surface, spread as a film after 24 hours. However, the toxicant concentrations maintained in laboratory tests, rarely occur for any length of time in the sea and crude oils vary greatly in their toxicity. Losses due to evaporation and rapid dilution of soluble compounds ensures that toxic effects are not prolonged. Smith (1968) has shown that dilution of oil spillage occurs rapidly on the open shore.

There is abundant evidence that freshly spilled crude oils contain low-boiling point substances which are acutely toxic to planktonic organisms and the most vulnerable are the 'sensitive crustaceous plankton' (Johnston, 1977). The most toxic components appear to be the water soluble aromatic derivatives. The aromatic solvents found in early emulsifiers were also found to be highly toxic to planktonic organisms at emulsifier levels of less than 1 mg l^{-1}.

The effects of an oil/dispersant mixture

Conner (1972) has shown that oil is the important toxic component when mixed with low-toxicity dispersants. The non-ionic oil dispersant we tested mixed with oil is rapidly biodegraded although the crude oil dispersed as droplets and oil in water emulsion requires a different range of micro-organisms for degradation.

Toxicity tests in general involve long pollutant administration periods but there is a need to test the resistance of organisms to a peak concentration which is subsequently reduced. The concentrations of pollutant(s) which are toxic under natural conditions are not readily derived from standard toxicity tests which only serve to rank substances in order of toxicity. The question of whether monitoring any of the various metabolic parameters can provide information on the direct effects of sub-lethal quantities of pollutants has been partly answered. Although minor changes in heart and gill ventilation rate and oxygen consumption occurred with quite small concentrations of some substances, major changes did not occur until pollutant concentrations reached lethal levels. Most of the changes monitored here were only associated with a temporary impairment of metabolic function which was compensated for relatively quickly.

Therefore, measurements of cardiac and ventilatory activity and oxygen consumption are not particularly useful as sensitive indicators of stress. However, results are provided quickly with few animals, and the method is particularly appropriate where organisms are likely to be exposed only briefly to a certain pollutant.

Acknowledgements We thank R.G.J. Shelton, P.D. Anthony, C. Robb and C.D. Hall for their help and technical assistance. Thanks are also due to B.B. Parrish and D. Whitford for their comments on the manuscript.

Summary Cod, *Gadus morhua L.*, were exposed separately to waste from an aluminium smelter (cryolite recovery sludge), a solution of an oil dispersant (BP 1100 WD), crude oil from the North Sea (Forties Field) and a mixture of oil and dispersant in sea water. During exposure to some of the pollutants, changes in heart rate, gill ventilation rate and amplitude, and oxygen consumption were evident. Coughing was elicited on exposure to a dilution of whole cryolite recovery sludge but other changes were small. Gill ventilation rate increased dramatically when the fish were exposed to oil, dispersant and an oil/dispersant mixture. However, significant changes in heart rate, gill ventilation rate and oxygen consumption did not occur until pollutant concentrations were close to lethal levels. Therefore, the parameters monitored were not especially useful as sensitive indicators of stress but the method is valuable for examining the physiological effects of brief exposure to certain pollutants.

References

Abel, P.D. 1974. Toxicity of synthetic detergents to fish and aquatic invertebrates. *Journal of Fish Biology* **6**, 279-298.

Anderson, J.W., Neff, J.M. and Petrocelli, S.R. 1974. *Khan, M.A.Q. and Bederka, J.B. eds. Survival in toxic environments,* New York, Academic Press, 83-121.

Brown, G.W. 1976. Effects of polluting substances on enzymes of aquatic organisms. *Journal of the Fisheries Research Board of Canada.* **33**, 2018-2022

Calabrese, A., Thurberg, F.P., Dawson, M.A. and Wenzloff, D.R. 1975. Sublethal physiological stress induced by cadmium and mercury in the winter flounder, *Pseudopleuronectes americanus. Koemann, J.H. and Strik, J.J.T.W.A. eds. Sub-lethal effects of toxic chemicals on aquatic animals.* Amsterdam, Elsevier Scientific Publishing Company, 15-21.

Carlson, R.W. and Drummond, R.A. 1978. Fish cough response — A method for evaluating quality of treated complex effluents. *Water Research* **12**, 1-6.

Conner, P.M. 1972. Further investigations into the toxicity of oil and dispersants. *International Council for the Exploration of the Sea,* CM E:14, 1-4 (mimeo).

Cormack, D. and Nichols, J.A. 1977. The natural and chemical dispersion of oil in the sea. *Rapport et Procès-Verbaux des Réunions. Conseil International pour l'Exploration de la Mer*, **171**, 97-100.

Dively, J.L., Mudge, J.E., Neff, W.H. and Anthony, A. 1977. Blood PO_2, PCO_2 and pH changes in brook trout (*Salvelinus fontinalis*) exposed to sublethal levels of acidity. *Comparative Biochemistry and Physiology,* **57A**, 347-351.

Holeton, G.F. 1971. Oxygen uptake and transport by the rainbow trout during exposure to carbon monoxide. *Journal of Experimental Biology,* **54**, 239-254.

Hughes, G.M. 1975. Coughing in the rainbow trout (*Salmo gairdneri*) and the influence of pollutants. *Revue Suisse de Zoologie*, **82**, 47-64.

Hughes, G.M. and Adeney, R.J. 1977a. The effects of zinc on the cardiac and ventilatory rhythms of rainbow trout (*Salmo gairdneri*, Richardson) and their responses to environmental hypoxia. *Water Research,* **11**, 1069-1077.

1977b. Variations in the pattern of coughing in rainbow trout. *Journal of Experimental Biology,* **68**, 109-122.

Johnston, R. 1977. What North Sea oil might cost fisheries. *Rapport et Procès-Verbaux des Réunions. Conseil International pour l'Exploration de la Mer,* **171**, 212-223.

Kiceniuk, J.W., Penrose, W.R. and Squires, W.R. 1978. Oil spill dispersants cause bradycardia in a marine fish. *Marine Pollution Bulletin,* **9**, 42-45.

Kühnhold, W.W. 1977. The effect of mineral oils on the development of eggs and larvae of marine species. *Rapport et Procès-Verbaux des Réunions. Conseil International pour l'Exploration de la Mer*, **171**, 175-184.

Lønning, S. 1977. The effects of crude Ekofisk oil and oil products on marine fish larvae. *Astarte* **10**, 37-47.

Lunn, C.R., Toews, D.P. and Pree, D.J. 1976. Effects of three pesticides on respiration, coughing and heart rate of rainbow trout (*Salmo gairdneri*, Richardson). *Canadian Journal of Zoology* **54**, 214-219.

Moore, S.F. and Dwyer, R.L. 1974. Effects of oil on marine organisms: a critical assessment of published data. *Water Research* **8**, 819-827.

Morgan, W.S.G. and Kühn, P.C. 1974. A method to monitor the effects of toxicants upon breathing rate of largemouth bass (*Micropterus salmoides* Lacepede). *Water Research* **8**, 67-77.

Schaumberg, F.D., Howard, T.E. and Walden, C.C. 1967. A method to evaluate the effects of water pollutants on fish respiration. *Water Research* **1**, 731-737.

Sellers, C.M., Heath, A.G. and Bass, M.L. 1975. The effect of sub-lethal concentrations of copper and zinc on ventilatory activity, blood oxygen and pH in rainbow trout (*Salmo gairdneri*). *Water Research* **9**, 401-408.

Skidmore, J.F. 1970. Respiration and osmoregulation in rainbow trout with gills damaged by zinc sulphate. *Journal of Experimental Biology* **52**, 481-494.

Smith, J.E. 1968. 'Torrey Canyon' pollution and marine life. Cambridge University Press, 196 pp.

Sparks, R.E., Cairns, J. and Heath, A.G. 1972. The use of bluegill breathing rates to detect zinc. *Water Research* **6**, 895-911.

Sprague, J.B. 1971. Measurement of pollutant toxicity to fish — III. Sublethal effects and safe concentrations. *Water Research* **5**, 245-266.

Thomas, R.E. and Rice, S.D. 1975. Increased opercular rates of pink salmon (*Oncorhynchus gorbuscha*) fry after exposure to the water-soluble fraction of Prudhoe Bay crude oil. *Journal of the Fisheries Research Board of Canada* **32**, 2221-2224.

Van Dam, L. 1938. On the utilisation of oxygen and regulation of breathing in some aquatic animals. PhD Thesis, Gröningen University, Netherlands.

Zeitoun, I.H. 1977. The effect of chlorine toxicity on certain blood parameters of adult rainbow trout (*Salmo gairdneri*). *Environmental Biology of Fishes* **1**, 189-195.

Appendix
Composition of cryolite recovery sludge

The alumina and carbon in the complex dilute aqueous waste are derived from waste cell electrode material leached with caustic soda to remove the bulk of the cryolite (sodium aluminium fluoride). The solids can amount to 200 g l^{-1} but the two samples supplied gave about 70 to 80 g l^{-1}. An approximate analysis of the dry solids gave:—

	% Total Solids
Alumina	50 to 70
Carbon	25
Cryolite	3 to 15

Others (mainly iron 1,000—2,000 mg l^{-1}, nickel 50-250 mg l^{-1}, vanadium 20-40 mg l^{-1} with some zinc and lead oxides) — all as slurry concentrations.

The liquid contains small amounts of neutral sodium and calcium salts together with trace amounts of the following (in mg l^{-1}).

Iron	25
Cadmium	< 1
Lead	< 1
Zinc	< 1
Chromium	< 5
Nickel	< 5
Ammonia	< 20
Cyanide (free)	50
Cyanide (complexed)	100
Fluoride	1 to 2
Oil/grease (persistent)	< 10
Oil/grease (non-persistent)	< 10
Benzene-soluble matter	40

The analyses of the cryolite recovery sludge are derived from the declared composition of the sludge submitted in respect of the application in June, 1975, for licence under the Dumping at Sea Act, 1974, together with details relating to two samples sent for toxicity testing.

Following comments from DAFS, the liquor is now submitted to further treatment to destroy the cyanides. The free cyanide content of the two samples was 4 mg l^{-1} (Sample 1) and 11 mg l^{-1} (Sample 2).

RE 77058 750 8/80 TCL

PREVIOUS SCOTTISH FISHERIES RESEARCH REPORTS

1975

No. 1. The Aberdeen Single-Anchor Mooring.
By R. Payne, 7 pp.

No. 2. The Aberdeen Shallow Water Current Meter Mooring.
By R. Payne, 13 pp.

1976

No. 3. The Bridger Version of the Gulf III High Speed Plankton Sampler.
By J.A. Adams, 42 pp.

No. 4. Some Parasites of Plaice *Pleuronactes platessa* L. in Three Different Farm Environments.
By K. McKenzie, A.H. McVicar and I.F. Waddell, 14 pp.

No. 5. Simulation of Vertical Structure in a Planktonic Ecosystem.
By J.H. Steele and E.W. Henderson, 29 pp.

No. 6. The Intertidal Fauna of Sandy Beaches – a Survey of the Scottish Coast.
By A. Eleftheriou and A.D. McIntyre, 61 pp.

No. 7. A Net Drag Formula for Pelagic Nets.
By A.J. Reid, 12 pp.

1977

No. 8. The Aberdeen Sedimentation trap and its Moorings.
By R. Payne and J.M. Davies, 11 pp.

No. 9. A Preliminary Model of Shear Diffusion and Plankton Populations.
By G.T. Evans, J.H. Steele and G.E.B. Kullenberg, 20 pp.

No. 10. Diving Observations on the Efficiency of Dredges used in the Scottish Fishery for the Scallop, *Pecten maximus* (L.).
By C.J. Chapman, J. Mason and J.A.M. Kinnear, 16 pp.

1978

No. 11. Comparative Fishing for Flatfish using a Beam Trawl fitted with Electric Ticklers.
By P.A.M. Stewart, 10 pp.

No. 12. The Value of Direct Observation Techniques by Divers in Fishing Gear Research.
By J. Main and G.I. Sangster. 15 pp.

1979

No. 13. The Occurrence of Plerocercoids of Diphyllobothrium spp. in Wild Cultured Salmonids from The Loch Awe Area.
By R. Wootten and John W. Smith, 8 pp.

No. 14. A study of Bottom Trawling Gear on both Sand and Hard Ground.
By J. Main and G.I. Sangster, 15 pp.

No. 15. The Coastal Movements of Returning Atlantic Salmon, *Salmo salar* L.
By A.D. Hawkins, G.G. Urquhart and W.M. Shearer, 15 pp.

No. 16. Hydrodynamic Characteristics of Trawl Warps.
By D.N. MacLennan. 10 pp.

No. 17. Some Parasites and Diseases of Blue Whiting, *Micromesistius poutassou* (Risso), to the North and West of Scotland and at the Faroe Islands.
By K. MacKenzie, 14 pp.